Investigating the Oxidoreductase Activity of Members of the Chloride Intracellular Ion Channel Protein Family

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Certificate of Original Authorship

I, Hala Mishaal Ali, certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged.

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Publications

Publications arising from this thesis

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Conference Presentations

Poster presentations

1- Hala M Ali, Khondker R Hossain, Heba Al Khamici, Lele Jiang, Stella M Valenzuela (**2016**) Members of the Chloride Intracellular Ion Channel Protein Family Demonstrate Cell Protective Effect Against Oxidative Stress New Horizons, Sydney, Australia.

2- Hala M Ali, Khondker R Hossain, Stella M Valenzuela (**2017**) Members of the Chloride Intracellular Ion Channel Protein Family Catalyse Protein Deglutathionylation. New Horizons, Sydney, Australia.

3- Hala M Ali, Khondker R Hossain, Stella M Valenzuela the 35 th Combined Health Science Conference, **New Horizons 2018**: 19-20 November 2018 at The Kolling Institute. Members of the Chloride Intracellular Ion Channel Protein Family Catalyse Protein Deglutathionylation.

Oral presentations

1- Hala M Ali, Khondker R Hossain, Stella M Valenzuela **ComBio2018**: 23 - 26 September 2018, International Convention Centre Sydney, Darling Harbour. Members of the Chloride Intracellular Ion Channel Protein Family Catalyse Protein Deglutathionylation.

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List of Abbreviations

AsA	Ascorbic Acid
Å	Angstrom
Arg	Arginine amino acid
Asn	Asparagine amino acid
ATCC	American Type Culture Collection
BCA	Bicinchoninic acid assay
BSA	bovine serum albumin
°C	centigrade
CaCCs	Calcium activated Chloride channel
CaCl2	calcium chloride
CAFs	Cancer-Associated Fibroblast secretome
Car	Carbenicillin
CDCs	Cholesterol-Dependent Cytolysins
C-domain	Carboxy terminal domain
cDNA	complementary Deoxyribonucleic acid
CFTR	Cystic fibrosis transmembrane conductance regulator
CHO-k1	Chinese Hamster Ovary cells
Chol	Cholesterol
CIC	Chloride Ion Channel
CLIC	Chloride Intracellular Ion Channel
Cl^-	Chloride ion
CLIC (WT)	Chloride Intracellular Ion Channel protein (wild type)
Cys	Cysteine
CV	column volumes
COPD	Chronic obstructive pulmonary disease
Da	Dalton
DDT	Dithiotheritol
DHA	Dehydroascorbate

DHAR	Dehydroascorbic acid reductase
DMSO	Dimethyl sulfoxide
Dm CLIC	*Drosophila melanogaster* CLIC protein
DNA	Deoxyribonucleotides acid
DIDS	4, 4'Di isothiocyano-2, 2'-stilbenedisulfonic acid
ECM	extracellular matrix
EDTA	ethylene diamine tetra acetic acid
E-coli	*Escherichia coli*
EPR	Electron Paramagnetic Resonance
ER	Endoplasmic Reticulum
ERK7	Extracellular signal-regulated kinase 7
EXC	Excretory Canal abnormality
EXL	EXC4-like
FRET	Fluorescence Resonance Energy Transfer
3D	three dimensions
GABA	Gamma-Amino Butyric Acid
Glu	Glutamic amino acid
Grx	Glutaredoxin
GR	glutathione reductase
GSH	Reduced glutathione
G-site	Glutathione binding site
GST	Glutathione S-transferase
HEDS	2-hydroxyethyl disulphide
His	Histidine
H_2O_2	hydrogen peroxide
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
H2O	water
IAA	Indanyloxyacetic acid
IPTG	Isopropyl-thio-β-D-galactopyranoside
Kan	Kanamycin
KCl	potassium chloride

KH_2PO_4	potassium dihydrogenphosphate
K_2HPO_4	dipotassium hydrogenphosphate
LB	Luria broth
Lys	Lysine amino acid
MAP kinase	Mitogen Activated Protein kinase
µm	micro molar
NAD+	nicotinamide adenine dinucleotide
NaOH	sodium hydroxide
NaCl	sodium chloride
NADPH	reduced nicotinamide adenine dinucleotide phosphate
Na_2HPO_4	disodium hydrogen phosphate
NCC27	Nuclear Chloride Channel protein-27 KDa
NEB	New England Biolabs
Ni^{2+} ions	nickel ions
NTA	nitrilotriacetic acid matrix
N-domain	Amino terminal domain
NMR	Nuclear Magnetic Resonance
O.D.	optical density
OH•	hydroxyl radical
ORF	open reading frame
P64	bovine chloride channel protein
PFT	Pore Forming Toxin
PBS buffer	Phosphate-buffered saline
PICOT	protein kinase C interacting cousin of thioredoxin
Phe	Phenylalanine amino acid
PTMD	Putative Transmembrane Domain
Pro	Proline amino acid
PLB	Planner Lipid Bilayer
PVDF	polyvinylidene fluoride membrane
PTM	post-translational modification
RT	room temperature

RT-PCR	Real-time quantitative Polymerase Chain Reaction
RNR	ribonucleotide reductase
RyR	Ryanodine receptor
ROS	Reactive Oxygen Species
SDS-PAGE	Sodium Dodecyl Sulfate -Polyacrylamide
Ser	Serine amino acid
SEC	Size Exclusion Chromatography
TBE Buffer	tric,boric acis, ethylene diamine tetra acetic acid buffer
TCEP	tris-2-carboxyethyl-phosphine
TEMED	NNNN'-tetramethylethylenediamine
TGM2	transglutaminase-2
Trp 35	Tryptophan 35 in CLIC1
TMD	Transmembrane Domain
Trx	Thioredoxin
TrxR	thioredoxin reductase
UV	Ultraviolet
Val	Valine amino acid
WCLs	Whole Cell Lysates
X	any amino acid

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Abstract

The chloride intracellular ion channel (CLICs) proteins are atypical anion selective channel proteins, as they are principally soluble proteins, with some members now known to also demonstrate enzymatic activity. Structural studies demonstrate that the CLIC proteins share strong structural homology with members of the glutathione-S-transferase superfamily of enzymes, in particular the omega glutathione-S-transferase (GST-Ω) members.

The discovery that the CLIC proteins have the functional ability to act as glutathione dependent oxidoreductase enzymes, also suggested a role for them as cell protective proteins and antioxidants. Therefore, this PhD project aimed to further define the functional activity of the CLIC proteins, in particular CLIC3 as one of more recently identified members.

The principal findings of this PhD project include demonstrating for the first time that CLIC3 has glutathione dependant oxidoreductase activity via its dithiol active enzymatic site. In this study, we directly contributed to the finding that the extracellular activity of transglutaminase-2 (TGM2) is regulated by CLIC3's oxidoreductase activity, and their interaction was dependent upon the redox environment. Furthermore, our *in vitro* studies of CLIC3, were key in helping demonstrate a critical role for secreted CLIC3 in cancer metastasis and tumour cell invasiveness.

To further investigate this oxidoreductase activity of the CLIC proteins we used a bacterial cell model to probe their ability to protect cells against oxidative assault. Recombinant CLIC proteins were expressed in bacterial *E. coli* cells, followed by their exposure to the oxidising agent hydrogen peroxide (H2O2). Expression of CLIC1 by the *E. coli* cells was found to provide increased tolerance of up to 5mM H2O2, while CLIC3

afforded some protection, with no difference seen for cells expressing CLIC4. This work for the first time demonstrates CLIC1 protein acting in antioxidant cellular protection.

The final part of this PhD project pursued the study of the CLIC proteins' glutathionylation activity. Given their close resemblance to the GST-omega-1 proteins, we hypothesised that members of the CLIC family would like them, be capable of deglutathionylation activity. Using a synthetic peptide substrate, our *in vitro* studies demonstrate members of the CLIC family have significant deglutathionylation activity supporting a major role for them in the cellular gluathionylation cycle.

In conclusion, our results provided new insights into the functions of the CLIC proteins as soluble enzymes, with functions in cellular antioxidant protective mechanisms. Most importantly, it points to their role as post-translational regulators of target proteins via their deglutathionylation activity.